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METHODS IN QUANTITATIVE RESEARCH ON MARINE MICROPLANKTON

BY
WINFRED EMORY ALLEN

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INTRODUCTION

On January 2, 1929, was begun the tenth year of daily collections of microplankton by the Scripps Institution of Oceanography at its own pier and at Pt. Hueneme pier more than one hundred miles farther north. All of the collecting in the regular series at these stations had been done by dipping about twenty-five liters of water from the surface of the sea and filtering it through No. 25 bolting silk, having mesh openings thirty to one hundred micra wide in the new condition, somewhat less as shrunken for and by regular use, and increasingly less as clogging and spreading of threads progress with use.

Since preliminary experiment showed this method to be superior to any other filtration method, and much more facile than any concentration method, for work with plankton diatoms under obligatory conditions of operation, it was adopted as standard for a ten-year period without extensive effort to estimate the percentage of losses of organisms through the filtration openings. Preceding experience led to an assumption that not more than 50 per cent of the plankton diatoms present in the water filtered would be lost, and that the loss would be negligible more often than it would be so great as that. It

was supposed that losses of very small diatoms, dinoflagellates, and many other constituents of the microplankton of small dimensions would be indefinitely great.

With the beginning of collecting for the tenth year, it seemed desirable to start at the Institution pier a parallel series of catches by a concentration method in order to obtain positive information concerning the extent and character of losses through the filtration net. Gravity settling was selected as the method most satisfactory for concentration, and dispensing burettes of one-liter capacity were tried for a few days. They proved unsatisfactory because of leakage and because the slopes interfered with close concentration of the organisms. A series of graduated cylinders was then placed in permanent use, transfer from the first, of liter capacity, to those smaller, being made by pouring after decanting the surplus fluid. There is more or less loss of very light organisms in decanting but it is impossible to prevent all loss without very elaborate precautions not warranted by the purposes of the investigation.

The comparison series is being run through the whole year of 1929 in order to observe differences in catches by the two methods according to numbers and kinds of components. For the sake of being sure that the settling method would yield material satisfactory for the purposes of comparison, detailed counts were made of catches for the first half of January, 1929. The results of this short study have indicated certain conditions which it seems worth while to discuss now because it will probably be three or four years before a report will be completed for the whole year.

For the comparison catches (as for ordinary routine catches) the initial sample of water is dipped from the surface of the sea with a common pail of galvanized iron. About twenty-eight liters are thus obtained and mixed together in a large crockery jar with a drain cock at the bottom. For the secondary samples, one liter is dipped from the mixed water for gravity concentration, and a little more than twenty-five liters is drained through the filtration net. The one liter is preserved in a roughly modified Bouin's fluid consisting of formaldehyde saturated with picric acid and supplemented with acetic acid. The twenty-five liter sample is preserved in formaldehyde. In the January studies the small sample was diluted to ten milliliters and the large sample to one hundred milliliters for microscopic examination and enumeration.

CYLINDRICAL GRADUATES

Gross results of study of twelve catches obtained by using cylindrical graduates are exhibited in table 1, where they are compared with the results of study of standard filtration catches. Certain features of the relations of the two methods to catches of different species are shown in table 2 for three species of diatoms and one species of dinoflagellate.

Gravity settling in graduates was used instead of centrifuging, partly because of personal preference but mainly because of greater convenience in handling a liter of water under local conditions. From

TABLE 1
COMPARISON OF SETTLING AND FILTRATION CATCHES
LA JOLLA PIER, 1929

A. Diatoms per liter

Haul number	SETTLING			FILTRATION NET		
	Species	Occurrence	Cells	Species	Occurrence	Cells
2915	12	1,500	3,150	9	700	2,640
2916	5	500	1,650	11	480	2,720
2917	19	7,900	35,750	22	6,260	37,560
2918	22	6,300	38,050	26	6,320	41,240
2919	26	9,600	53,250	28	8,420	41,160
2920	23	11,100	52,450	29	8,240	38,800
2921	28	16,250	71,150	26	12,560	66,520
2922	20	14,950	46,400	21	9,400	40,960
2923	20	16,000	51,400	24	9,360	36,420
2924	14	5,300	15,200	11	1,740	7,140
2925	13	2,950	9,450	12	1,140	4,200
2926	8	850	2,200	11	480	1,400

B. Dinoflagellates per liter

2915	5	5,800	5,800	8	660	660
2916	4	1,400	1,400	6	600	600
2917	5	2,450	2,450	6	380	380
2918	7	4,850	4,850	8	420	420
2919	8	7,800	7,800	11	1,040	1,040
2920	11	7,950	7,950	10	660	660
2921	6	4,450	4,450	10	480	480
2922	8	12,300	12,300	6	380	380
2923	7	3,550	3,550	11	700	700
2924	6	850	850	6	220	220
2925	6	1,600	1,600	6	200	200
2926	4	700	700	5	180	180

extensive experience with both methods of concentration, I am convinced that they are equally good and that there is no demonstrable difference in their results when properly used. Where there is no limitation of time and where the material can be kept free from dis-

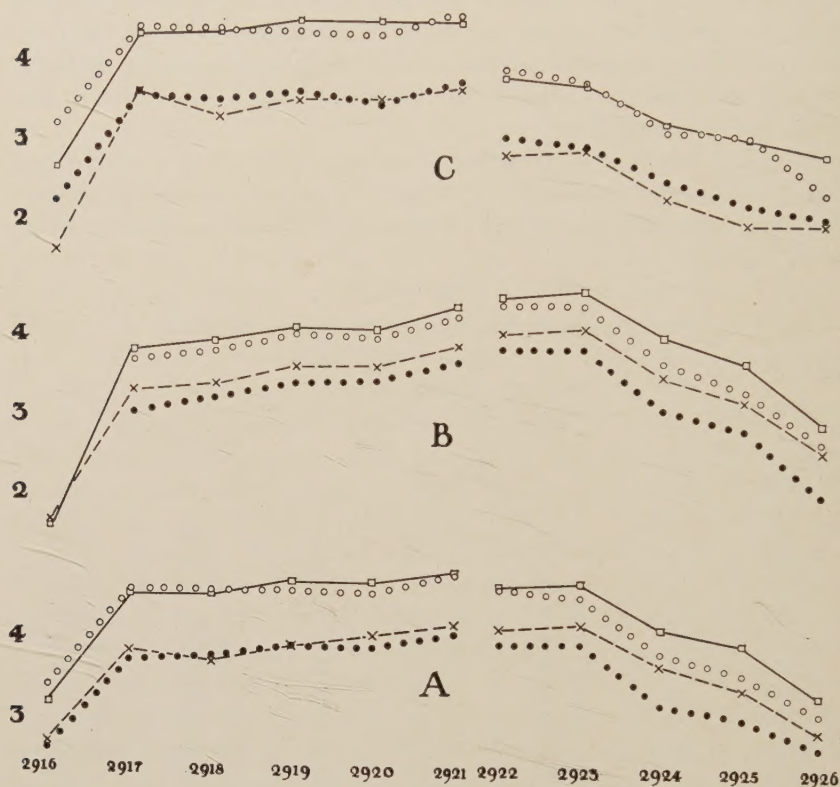


Fig. 1. Graphs to show distribution by logarithms of total occurrences and of total cells of diatoms caught by methods of settling and of filtration.

A. Total diatoms. B. *Nitzschia seriata*. C. Genus *Chaetoceras*.
 ○ ○ ○ ○ Filtration cells.
 Filtration occurrences.
 □ — □ Settling occurrences.
 x — x Settling cells.

turbance, settling has the important advantage of low cost of equipment and maintenance and of freedom from care for and dependence upon machinery. Where time for completing a catch is limited and where conditions are disturbing, the centrifuge method may be obligatory if one would avoid all filtration devices.

TOTAL DIATOMS

The catches by graduate consistently showed higher numbers of occurrences of diatoms per liter than did the filtration catches (table 1). The numbers of species were mostly smaller. But nine out of twelve gravity catches showed larger (mostly much larger) numbers of cells per liter than were found in the corresponding filtration catches.

It so happened that the twelve catches (daily except for the two Sundays missed) extended through a period of rise and fall in abundance (a pulse) of diatoms. The general appearance of table 1 indicates that the peak of the pulse was reached at about the middle of the group of catches, and this appearance is supported by the shape of the polygon (fig. 1A) based on logarithms of the numbers recorded,

TABLE 2

A. <i>Nitzschia seriata</i> Cl.					B. Genus <i>Chaetoceras</i>			
Haul number	SETTLING		FILTRATION No. 25 SILK		SETTLING		FILTRATION No. 25 SILK	
	Occur- rences	Cells	Occur- rences	Cells	Occur- rences	Cells	Occur- rences	Cells
2915	100	100			300	1,550	540	2,140
2916	50	50			50	450	180	1,740
2917	2,100	7,550	1,220	5,140	5,050	23,650	4,360	30,100
2918	2,600	7,800	1,800	7,280	2,150	26,500	3,560	30,880
2919	4,600	14,800	2,640	11,080	3,600	34,800	4,720	27,060
2920	5,000	13,800	3,120	10,080	3,950	32,900	3,720	24,820
2921	8,600	28,300	5,600	21,040	5,250	36,050	5,800	41,760
2922	12,400	36,200	7,520	29,440	700	6,400	1,200	8,640
2923	13,400	41,400	7,760	28,320	800	5,550	880	6,280
2924	3,550	11,350	1,320	5,520	200	1,750	320	1,480
2925	1,950	5,650	780	2,420	100	1,150	160	1,300
2926	400	850	120	500	100	700	100	240
C. <i>Pseudoeunotia doliolum</i> W.					D. <i>Prorocentrum micans</i> E.			
2915	200	550			100		20	
2916	250	1,000	100	440	350		120	
2917	250	800	140	720	650		40	
2918	300	1,200	100	520	800		100	
2919	150	650	140	800	950		40	
2920	200	900	140	680	550		40	
2921	300	900	40	320	600		80	
2922	500	2,550	100	560	550		0	
2923	150	800	40	120	150		40	
2924	50	200	0	0	200		20	
2925	300	1,450	20	120	350		0	
2926	0	0	60	380	250		20	

although in this polygon the peak is not very prominent in relation to several other catches. Not only has the use of logarithms in the graph reduced the prominence of the peak, but it has also reduced the prominence of differences incident to the two methods of collecting, so that all four polygons have a marked similarity in general form.

Concerning the two methods of collecting, this similarity is much stronger in the first six catches of the true pulse (beginning with catch 2916) than it is in the last five, probably because there were, actually and relatively, more representatives of forms having prominent lateral setae in the earlier catches, where they helped to entangle other forms as well as their own kind in the net. (Cf. tables 1 and 2 and figures 1A, 1B, 1C.)

LEADING GROUPS

So far as this short series is concerned there were two genera showing notable prominence, *Chaetoceras*, mainly represented by two species but having several others present, and *Nitzschia*, represented almost entirely by one species but having another present. *Chaetoceras* (fig. 1C) overshadowed *Nitzschia* (fig. 1B) in the first week but fell to the inferior position in the second week, so that the two combined to keep the polygon of total diatoms (fig. 1A) at fairly even height through two-thirds of the pulse. In other words, the single pulse for total diatoms was really a composite of a considerable number of species and genus pulses, most of which we must ignore for the sake of brevity and clarity, but two of which require further attention, together with one other selected for its peculiar showing.

Genus *Chaetoceras*

The genus *Chaetoceras* is notable for two characteristics, namely, prominent setae, and filamentous grouping of cells into colonies of such form and size that a high percentage is caught by the No. 25 silk. The setae, on account of entanglement with other colonies and with fibrils of the silk, greatly increase the tendency to be caught. To greater or less extent these entanglements also aid in the capture of non-setose forms which might otherwise escape.

From the general appearance of *Chaetoceras* catches in table 2B and in graph C of figure 1, it may be assumed that concentration by settling and filtration by No. 25 silk (as conducted in this series) yield essentially similar results for species of this genus, the filtration method making a little better showing.

In passing, it should be noted that the difference in favor of the filtration net is mainly due to size of samples, the concentrate sample being too small consistently to exhibit the same proportions of small to large colonies that is to be expected in the much larger filtration sample. Indication of this is visible in the close resemblance of numbers in the columns for occurrences, which run so nearly alike that the individual differences might be included in the fairly wide range of error incident to micro-enumeration. In the columns for numbers of cells the slightly more favorable showing of the filtration catches is certainly caused by the larger sample's getting more large colonies than the small sample.

However, the error may run in the reverse direction if the small sample happens to include an exceptionally large colony. For example, in catches 2918 and 2919 (table 2B) a single larger colony of *Chaetoceras sociale* Laud. caused the superior showing of cells in the concentration catches.

Nitzschia seriata Cleve

This species is colonial but the colonies are usually small, not many including more than six cells. The cells are smooth and spindle-shaped, forming a colony by coherence of the tapered ends, which sometimes fit together in such a way as to give the colony the superficial appearance of a continuous filament, less than six micra wide more often than it is over that width. In spite of this small size its loss through the filtration net as indicated by comparison with the concentration catches (table 2A and fig. 1B) was lower than 50 per cent in none but the smallest catches of the series, where other errors may have been equally large on account of the scanty material. The showing for numbers of cells caught by net was still better, evidently because of influence of inclusion of a greater number of larger colonies in the larger samples.

Notwithstanding the considerable disparity in efficiency of sampling by the two methods, the polygons of distribution are remarkably similar in general form (fig. 1B), giving reason for supposing that, under the methods of statistical treatment (logarithms, averages, summary tables, etc.) obligatory for large series, the catches by the filtration net give fairly good approximations to relative values of numbers involved.

Pseudoeunotia doliolum Wallich

The non-setose cells of this colonial species are only slightly smaller than typical cells of *Nitzschia seriata* Cl. but they are joined side by side in small colonies in such a way that they offer a different type of resistance in the streamlets of water passing through the filtration openings. This may account for their greater percentage of loss by that method of collecting. Although no large numbers appeared in the series there was almost uniform superiority of numbers of cells in favor of the concentration catches (table 2C). The one exception was apparently due to a run of larger sizes of colonies in the filtration catch.

For species such as this, of small size and appearing in small numbers, the filtration net probably gives little information of value concerning numerical distribution.

DINOFLAGELLATES

In this series there were few dinoflagellates of the medium or larger sizes present. Of the smaller forms only *Prorocentrum micans* Ehr. was positively recognized as a dinoflagellate. Many or most of the others counted under that group name may have been zoospores, swarm spores, or flagellates of other types. They were grouped under a catch-all designation of "*Gymnodinium* spp." and recorded, partly for the sake of definite observation of their loss through the net, and partly because similar small organisms have been included in our standard records from time to time under that head.

As shown by comparing table 1B with table 2D these small forms of indistinct character overshadowed in numbers the recognized species of dinoflagellates, and their showing in the filtration catches was so erratic that no trend of distribution in the series is recognizable although the catches by graduate show a fairly plain tendency to increase through the first week.

The showing for *Prorocentrum micans* Ehr. is only a little better, so few of the small number present being retained by the filtration net that the errors of handling in the laboratory might account for most of the differences in different catches. Still, the indication of greater abundance in the first week is discernible, though less distinct than that given by the catches made by settling.

However, it seems to be clear that in periods of scant abundance the filtration net is not dependable for any kind of quantitative studies of *Prorocentrum* and other organisms of the same or smaller size. Of course, a tow net of the same silk would be still more unsatisfactory because of the many errors from other sources added to those of leakage through the filtration openings.

COMPARABILITY OF RECORDS

By far the most important indication in this short series concerns methods and units. Catches made by different methods are not, necessarily, comparable because they have been made with equal care. Methods satisfactory for handling one type of organism may be poor, or even valueless, for another. Catches made by different methods are not, necessarily, comparable because they are discussed in the same terms or because their contents are calculated in the same units. Nor are catches made by different methods necessarily comparable because they are given the same laboratory treatment; nor those taken from different volumes of water, if brought to the same volume for examination and enumeration. Calculations of abundance (density of population) of an organism cannot be completely satisfactory unless they are adapted to the character of the organism (particularly size), to the volume of water represented by the catch, to the dilution of the catch for examination (where fractional examination is made), to the size of fraction of the catch examined, and unless they are referred to an appropriate unit of distribution.

The two methods of collecting in this series were used by the same individual, with the same care, and in the same mixture of water. As a whole they seem comparable, in spite of considerable difference in aspect. Specifically, in respect to some of the components of the catches, they are not comparable, as is shown in the preceding details of discussion. If this be true of methods so nearly similar as these, how much more it is, or may be, true of dissimilar methods.

Apparently the genus *Chaetoceras* was satisfactorily handled in this short series (even at minimum abundance) by filtration of twenty-five liters of sea water through No. 25 silk and by the ordinary laboratory routine of dilution (from the preserved condition) to one hundred milliliters, followed by calculation of density of abundance from enumeration of one five-hundredth (or less) of the catch. But for *Nitzschia* the results were only tolerably satisfactory (especially in catches of light abundance), for *Pseudoeunotia* they were unsatisfactory, and for *Prorocentrum*, unreliable (all catches showing light abundance). On the other hand, the catches by settling (dilution ten milliliter for enumeration) were less satisfactory for the strongly colonial *Chaetoceras* (particularly in slight abundance) than they were for the single-celled forms. However, it seems possible that this last can be minimized or avoided by concentration of catches from small samples to proportionately smaller quantities for microscopic examination, or by enumeration of a larger part of the catch, or by both.

In 1928 I enumerated diatoms in a short series of samples taken where abundance was so great that a single milliliter of water as dipped from the sea showed enormous numbers of them. Except for the lack of any dilution, filtration, or concentration of the catch, I treated these samples according to my ordinary routine laboratory procedure in enumeration and in subsequent calculation to numbers per liter, making the actual count over only one-eighth of a milliliter. By this proceeding, if a species was recorded but once in the count it would appear as eight thousand to the liter in the final estimate. If a colonial species were represented by a single occurrence with a colony of one hundred cells, in the enumeration it would appear as eight hundred thousand cells per liter in the final estimate, although no other colony so large as that may have been present within a radius of a meter. On the contrary, of course, a sample so small might show no representatives of several species which were really fairly abundant in the surrounding water. In this particular series a number of samples were taken in the general vicinity and their close resemblance served to offset this type of error. But that does not mitigate the risk of comparing results of examination of extremely small samples with results from relatively very large samples in terms of units appropriate only to the larger sample, especially when the catches are solitary or disconnected in either time or space.

CONCLUSION

1. The study of this series shows positively that no method of *rapid* filtration (such as that through No. 25 silk) can give dependable results in quantitative investigations of the smaller diatoms and dinoflagellates, especially in periods of slight abundance. As a corollary to this statement, one should remember that slow filtration (such as that through taffeta, sand, or porcelain, is only practicable for very small samples of water, for which concentration methods (centrifuge or settling) are usually better.

2. For larger diatoms and dinoflagellates there is definite indication that filtration through No. 25 silk gives material dependable for general quantitative investigations, and that the filtration of measured quantities of five to twenty-five liters volume is practicable for such work.

3. There is strong evidence that catches made by gravity settling from one liter of water are fully dependable for most microplankton and that the method is highly practicable where conditions permit it to be used.

4. It appears that when certain precautions are taken the records of catches made by the settling method are comparable with those taken by the filtration method.

5. It is certain that many series of catches of plankton organisms taken by certain methods are not comparable with series taken by other methods (except, perhaps, in some of their grossest features). Therefore, in attempting comparisons it is usually necessary to give attention to the different methods of catching, to continuity of the series, to size of samples, to methods of computation, to units of computation, and to units of reference.

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